# 1.1 Introduction

## A. Purpose

The purpose of this document is to serve as a set of minimum standard protocols to be followed when carrying out health inspections on aquatic animals. These inspections may be used for intrastate, interstate, or international movement of animals. Using a standard set of procedures and protocols allows fisheries managers to better compare data and make better management decisions. However, the final decision to require the use of these protocols remains in the hands of those regulating bodies requesting/requiring health inspections. This handbook is in no way meant to provide, dictate, or supplant aquatic animal health policies of any entity and is solely intended to be a procedural handbook.

## B. Composition

This handbook reflects the combined efforts and expertise of the United States Fish and Wildlife Service Fish Health Centers and the American Fisheries Society Fish Health Section. It has been assembled by a vast array of individuals with academic and field expertise. It is a compilation of methodologies determined to be most appropriate for detecting the presence of specific pathogens during an aquatic animal health inspection. The methodologies have been taken from numerous sources, including the United States Fish and Wildlife Service's National Wild Fish Health Survey Manual, the 4$^{th}$ edition of the American Fisheries Society Blue Book, the 3$^{rd}$ edition of the Office International des Epizooties (OIE) Diagnostic Manual for Aquatic Animal Diseases, Alaska Department of Fish and Game, Fish Pathology Section Laboratory Manual, and the peer reviewed literature. Without the substantial contributions of these documents and the individuals that assembled and bench tested them, the development of this handbook may not have been possible.

## C. Design

To be effective and to meet the needs of what is a rapidly changing and expanding field, this handbook must be extremely dynamic. Every section, including this first chapter, is open to revision. The design of this handbook will allow it to grow and change as time progresses and standardized inspection techniques are required for additional pathogens or better testing methods are made available. The guidelines for introducing changes are outlined in Appendix 1 (A1). Appendix 3 (A3) summarizes the decision making process for the selected assays and pathogens so that future changes can be made efficiently.

## D. Selection of Pathogens

The pathogens considered in this handbook are those that have the potential to produce severe epizootics of clinical disease but are also known to exist in a carrier state. They are pathogens of regulatory concern, for which there are both screening and confirmatory tests available. The methodologies described herein are effective for the detection and identification of each pathogen in the absence of clinical signs.

## E. Selection Of Methodologies

The appropriateness of methodologies was determined based not only on performance characteristics (sensitivity, specificity, repeatability, and reproducibility) but also on the appropriateness in a given species or population, scientific acceptance and citable reference materials, cost, availability of reagents, availability of technology, time necessary to process samples and time required before a report can be issued, sample type and its viability, manpower requirements, number of samples to be done, the existence of reference standards, and safety.

The assays specified in this handbook are of two types. Generally, the screening method is one with a proven track record for isolation of a fish pathogen or, less commonly, for direct observation of a causative agent or detection of a component of a fish pathogen. In many cases (e.g. cell culture isolation of fish viruses), these were the first methods developed for this purpose. Although many of these assays have been used for sufficient periods of time to engender confidence in their utility, few if any of the screening methods have been subjected to the formal validation process outlined by the OIE (see "Validity of Chosen Methodologies"). Typically, this is because for many years, few competing assays were available for the purpose; however, there remains a need for the validation process to be applied to these screening tests. Nevertheless, specifying a single screening method ensures that the sensitivity of the assay used to detect a pathogen, while not know with precision, is relatively uniform wherever the assay is applied.

The second type of assay is a confirmatory, test used to verify the identity of a suspect agent. These assays are also in need of formal validation, but are used here only to accurately identify an agent isolated in culture or identified visually, with less regard as to their absolute level of sensitivity. Various assays may be appropriate as confirmatory tests if the specificity of each assay is high. Knowledge of the sensitivity of a confirmatory test becomes of increased importance when the assay is used to confirm a screening test that does not propagate the pathogen in culture. An example is the use of a DNA-based test (e.g. PCR) to confirm a serological test (e.g. FAT). In this case, both assays detect proxies for the actual agent. Better information on the sensitivity, specificity, repeatability and reproducibility of these assays would allow fish health workers to choose optimal combinations of such proxy assays.

## F. Validity of Chosen Methodologies

Validation is the evaluation of a process to determine its fitness for a particular use. For diagnostic assays, this generally involves measuring the ability of a certain test to accurately predict the infection status of an animal from which a sample was obtained. Naturally, many factors affect the ability of any assay to achieve absolute predictive power. In recent years, the development of assays based upon molecular approaches and the increased attention paid to issues of quality assurance have led to concerns about the relative performance of various diagnostic assays for fish diseases.

The principles involved in validation of diagnostic assays for infectious diseases are included as a chapter in the Diagnostic Manual for Aquatic Animal Diseases published by the Office International des Epizooties (OIE) in Paris, France. This material is available on the web (www.oie.int) and in hard copy from the OIE. In addition to presenting the principles involved, the OIE lists five stages for diagnostic assay validation and discusses these in some detail with references for further reading. The

chapter also describes the procedures by which the important parameters of sensitivity, specificity, repeatability, and reproducibility are determined within a known degree of statistical precision.

Finally, as new diagnostic tests for fish diseases become available, there is interest in comparing the relative performance of various assays. Formal validation methods provide a basis to accurately quantify the actual performance of assays used in fish health and provide information that can help to determine when newer assays should replace the established assays as screening or confirmatory methods. Validation methods can also resolve uncertainties as to the relative performance of materials from different commercial sources or molecular assays that are similar in type, but target different proteins or genes of a pathogen or that use different protocols or reagents. Unfortunately, few if any fish disease detection assays have ever been validated in this way. While validation testing involves much work, considerable expense, and the assembly of sets of standard samples of known infection status, the fish health community is very much in need of this information.

## G. Other Important Considerations

It should be noted that as with any inspection manual or handbook, the techniques employed provide only a snapshot in time. There is no guarantee that any inspected animal or population of animals is disease free. The only statement that can be made is that at the time the animals were sampled the disease organism was not detected by the testing methods utilized. Additionally, this handbook cannot cover every scenario that may occur. For this reason, it remains the obligation and responsibility of the individual inspector to determine what the best sampling protocols are for a given inspection and that any inspection methods or protocols used meet the requirements of the requesting entity.

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